

AD _____

Award Number: DAMD17-99-1-9564

Volume II

TITLE: Role of Angiogenesis in the Etiology and Prevention of
Ovarian Cancer

II: Angiogenesis Factors in the Malignant Transformation
of Ovarian Surface Epithelium

PRINCIPAL INVESTIGATOR: Sundaram Ramakrishnan, Ph.D.

CONTRACTING ORGANIZATION: University of Minnesota
Minneapolis, MN 55455-2070

REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are
those of the author(s) and should not be construed as an official
Department of the Army position, policy or decision unless so
designated by other documentation.

20040415 005

REPORT			Form Approved OMB No. 074-0188	
DOCUMENTATION PAGE				
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE October 2003		3. REPORT TYPE AND DATES COVERED Annual (1 Oct 2002 - 30 Sep 2003)
4. TITLE AND SUBTITLE Role of Angiogenesis in the Etiology and Prevention of Ovarian Cancer II: Angiogenesis Factors in the Malignant Transformation of Ovarian Surface Epithelium			5. FUNDING NUMBERS DAMD17-99-1-9564	
6. AUTHOR(S) Sundaram Ramakrishnan, Ph.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Minnesota Minneapolis, MN 55455-2070 E-Mail: sunnda001@tc.umn.edu			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES This is Volume II of IV				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited				12b. DISTRIBUTION CODE
13. ABSTRACT (Maximum 200 Words) <div style="margin-left: 40px;"> <p>Etiology of ovarian cancer is not completely understood. Previous studies have shown that both genetic and epigenetic factors could contribute to ovarian tumorigenesis. Epidemiological studies suggest that number of ovulation and ovulation related gonadotropic hormones could play a role in the malignant transformation of surface epithelium. In this project, we will investigate how the normal ovarian epithelium acquires tumorigenic phenotype. Specifically, the effect of angiogenic growth factors in the etiology of ovarian cancer is studied using a model system.</p> </div>				
14. SUBJECT TERMS Ovarian Cancer				15. NUMBER OF PAGES 7
				16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

Table of Contents

Cover.....	
SF 298.....	1
Table of Contents.....	2
Introduction.....	3
Body.....	3
Key Research Accomplishments.....	5
Reportable Outcomes.....	5
Conclusions.....	6
References.....	6
Appendices.....	6

(4) INTRODUCTION:

The vast majority of ovarian cancers arise from the surface epithelium. Only a small percent of ovarian cancers are genetically linked . Epidemiological data clearly suggest that ovulation related events can be associated with ovarian cancer development. Recurrent process of wounding and repair during repeated ovulation is implicated in transformation of surface epithelium. Transformed cells are then selected in vivo for their ability to attract new blood vessels, angiogenesis, one of the necessary steps involved in the establishment of tumors. Secretion of angiogenic factors is prognostically significant. Furthermore, gonadotropic hormones can induce angiogenic factors in cell cultures. These studies lead to a possible relationship between, ovulation, ovulatory hormones and angiogenic phenotype. Based on these data, the present proposal is designed to investigate the role of angiogenic factors in the development of ovarian cancer.

(5) BODY :

In our " Statement of Work" we proposed to perform Task # 1 and #2 during the first 36 months. Task 1 and 2 are related the expression of angiogenic growth factor in surface epithelium of the ovary . To evaluate whether angiogenic growth factor play a role in the development of ovarian cancer.

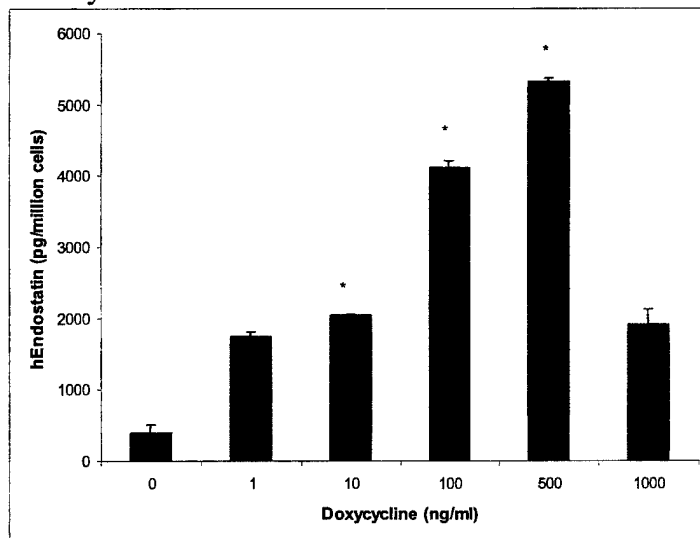
In the Year 3 report (2002) we described the tumorigenic potential of ROSE199 cells transfected with VEGF165. VEGF-ROSE cells formed tumors readily and formed malignant ascites when injected i.p. This model provided evidence for the role of VEGF in ovarian cancer development. Using this model we investigated whether neutralization of VEGF can revert the tumorigenic phenotype. In deed, treatment of mice with selective VEGF receptor specific kinase inhibitor, SU5416, inhibited tumor growth in nude mice.

During the last year (Year 4) , we investigated whether the angiogenic 'switch' of VEGF-ROSE cells can be balanced by genetically regulated expression of an antiangiogenic molecule. For this purpose, a tetracycline regulated expression cassette was generated. VEGF-ROSE cells were transduced with Tet-operated endostatin expression vector. Initial studies focused on tetracycline-induced expression of endostatin in vitro (Fig. 1) .

Results :

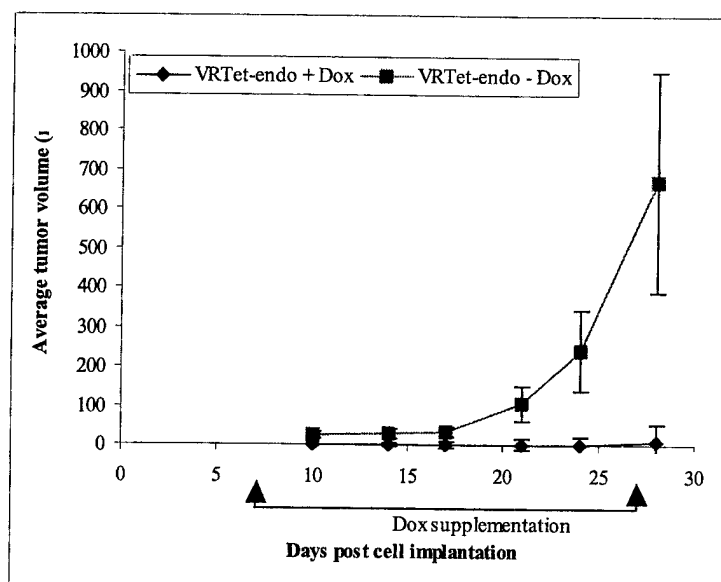
Fig. 1 shows the endostatin expression in VEGF-ROSE cells when treated with different concentrations of doxycycline. Up to 500 ng/ml of doxycycline there was a concentration-dependent stimulation of endostatin secretion as determined by a specific ELISA. At very high concentration, endostatin secretion was inhibited due to toxicity.

Fig. 1. Tetracycline-induced secretion of endostatin in VRTet cells.



After validating the that tet-operated expression of endostatin did not alter growth kinetics of transfected cells, we embarked on investigating the tumorigenic potential of this cell line. If proangiogenic phenotype of VEGF-ROSE cells were to be balanced by the secretion of endostatin, we expect reversal of tumorigenic phenotype. To test this hypothesis, athymic nude mice (20, female mice) were subcutaneously injected with VRTet-endo cells. Mice were then randomized into two groups of 10 each. After 7 days, one group of mice was treated with doxycycline in sucrose containing drinking water. The second group of mice served as a control. Tumor growth was followed by caliper measurements at regular intervals. Data in Fig. 2 show the tumor growth profile. Mice drinking regular water showed tumor growth reaching a volume of 700 mm³ by day 28. On the other hand the mice drinking doxycycline-containing water showed complete suppression of tumor growth. These studies clearly demonstrate that balancing the angiogenic potential of VEGF-ROSE cells by secretion of endostatin completely reverse the tumorigenic phenotype.

Fig. 2 . Doxycycline induced expression of endostatin completely suppresses VEGF-ROSE tumor growth in nude mice.



(6) **KEY RESEARCH ACCOMPLISHMENTS** :

VEGF-secreting ROSE cells were stably transfected with tetracycline inducible endostatin constructs.

Induced expression of endostatin was confirmed.

Growth kinetic of cells were compared to confirm no change in doubling rate in transduced cells.

Tumor growth inhibition by tetracycline-induced expression of endostatin was investigated.

(7) **REPORTABLE OUTCOMES** :

Manuscript in preparation summarizing these studies.

(9) **CONCLUSIONS** : Secretion of VEGF gives a selective advantage for non-tumorigenic ovarian surface epithelial cell line to become tumorigenic. Formation of malignant ascites is clearly associated with VEGF secretion, which is a characteristic of ovarian cancer. When angiogenic potential is balanced by the production of antiangiogenic molecule in the same cells, the tumorigenic potential was reverted. These studies suggest the important role of VEGF in ovarian cancer development. Furthermore, these studies provide evidence for the potential reversal of tumorigenic phenotype by co-expression of an antiangiogenic molecule, endostatin.

(10) **REFERENCES** :

(11) **APPENDIX** :